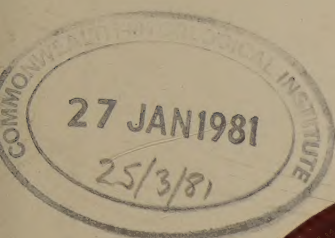


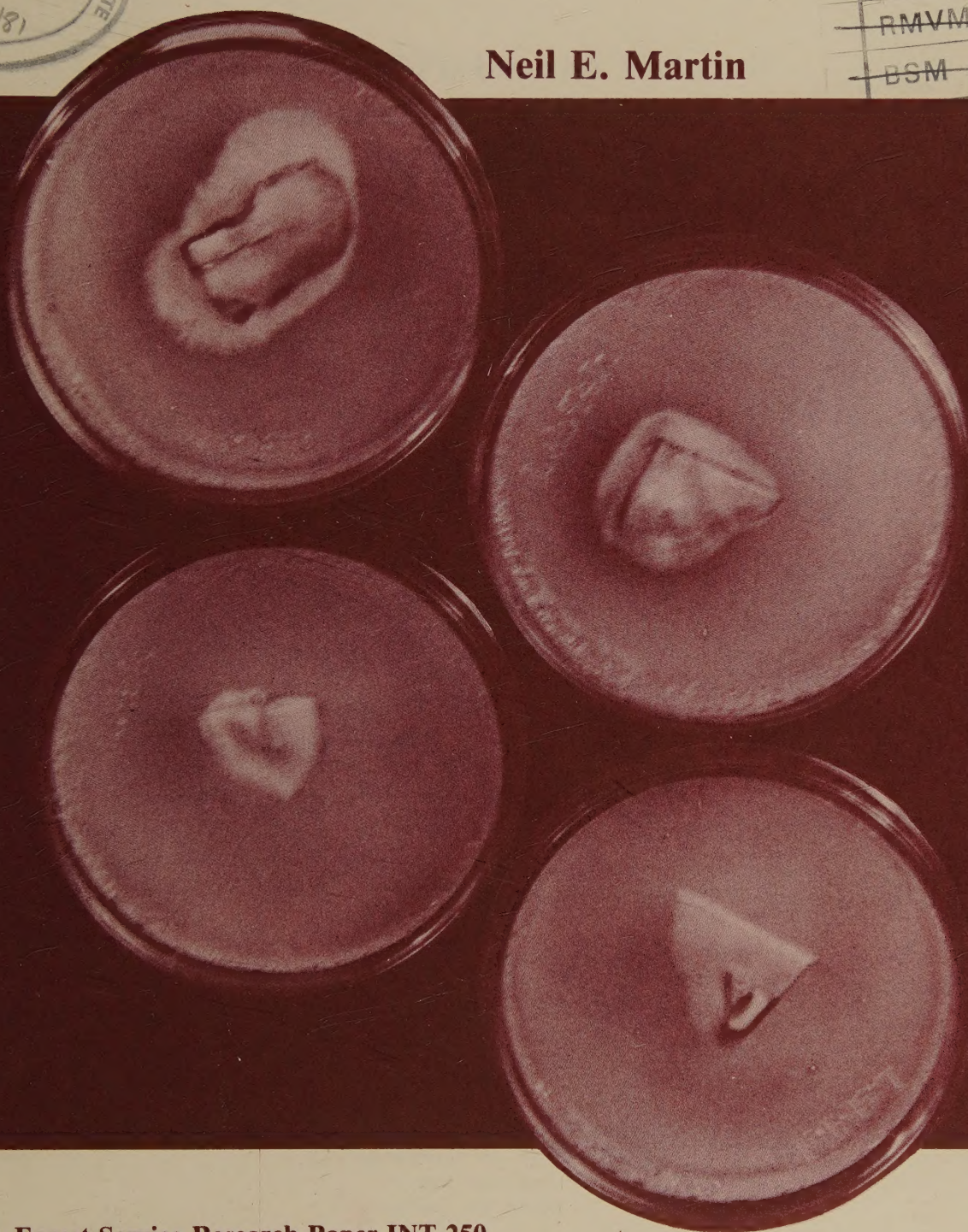
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Interactions of Pectins and Tissues of *Cronartium ribicola* and *Pinus monticola* in Culture



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**Interactions of Pectins and Tissues of
Cronartium ribicola and *Pinus monticola*
in Culture**

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NEIL E. MARTIN came to Intermountain Station in 1966 from a Ph.D. graduate program in plant pathology at Washington State University. He researched host-parasite interactions of blister rust attacks on western white pine until 1973. Since then he has primary responsibility for root pathogen research, with additional studies in dwarf mistletoe and blister rust problems.

RESEARCH SUMMARY

Growth of *Pinus monticola* and *Cronartium ribicola* in culture was sensitive to pectin compounds that may represent the products of enzymatic actions on pine pectins. Host's cells succumbed at lower concentrations of pectic compounds and were more sensitive as the complexity of the pectin compounds decreased than were the rust cells. The implications are that pectinase activities are controlled through the physiological conditions of the host cells and that the fungus can prolong the life of its host cells by utilizing the simpler pectic compounds that are detrimental to the host cells.

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INTRODUCTION

After a plant pathogen, such as a rust, enters its host, colonization and establishment are important to the continuation of its life cycle. *Cronartium ribicola*, the blister rust fungus, enters its pine host through needle stomata and from the substomatal cavity grows passively within intercellular spaces to colonize the host tissues. Intercellular fluids may provide substances required by this heterotroph, which would negate the necessity to enter the host cells. Instead, the fungus establishes itself by adhering to the exterior surfaces of host cells and then developing special hyphae, haustoria, that penetrate the host cell walls. Successful haustoria penetration occurs only when the rust fungus produces enzymes that are capable of hydrolyzing the cellulose, pectin, and protein constituents of the host cell.

Rust fungi are obligately parasitic and consequently dependent on the survival of their hosts, therefore, teleologically they must be weak pathogens with sensitively controlled host destroying mechanisms (Wood 1967). It follows that any enzyme systems used by the pathogen to modify and to penetrate the host cell walls must be highly discriminatory and operative only in a local area (Albersheim and Anderson-Prouty 1975). Studies using the electron microscope have shown the wall texture to be modified in the vicinity of contact between rust mycelium and pine cell walls (Welch and Martin 1975). Apparently the enzymes neither attack constituents that would kill the pine cell nor produce products in quantities that exceed detrimental threshold values for either the pathogen or host.

Earlier work illustrated the circumstantial evidence for the involvement of pectinases in the blister rust disease of western white pine (Martin 1967). Analysis of infected bark revealed a significant depression of pectic substances when compared to noninfected bark of the same tree (Welch and Martin 1974). The objective of the study reported here is to assess *in vitro* the tolerance of pine cells or rust mycelium to pectic compounds and the role of these substances in the host parasite relationship.

MATERIALS AND METHODS

Cultures of pine tissue were established on an agar medium as described by Harvey (1967). All explants used in this study were selected for uniformity in size, green color, and evidence of cell proliferation. Seven three-week-old cultures were submerged in 5 ml each of glass-distilled water solutions (0.001, 0.005, 0.01, 0.025, 0.05, 0.1, 0.5 percent concentrations) of citrus pectin, sodium polypectate, or pectic acid. The cultures were then observed at weekly intervals for continued proliferation, color changes, and signs of organ primordia.

Numerous isolates from 36 different infections of *C. ribicola* were established on agar media according to the procedures used by Harvey and Grasham (1969). Two isolates (the fastest and slowest growing) were selected for study (fig. 1).

The isolates were challenged with pectic compounds representing different molecular structural complexity by placing solutions of each compound in a small well cut into agar media (Dingle and others 1953). Six wells were positioned in a circle and spaced equidistant from the center of a petri dish and from each other. The wells were filled in the order of decreasing structural complexity with 1 percent aseptic solutions of citrus pectin, sodium polypectate, polygalacturonic acid, pectic acid, and glass-distilled water. Twenty-four hours later a 2 mm piece of agar cut from the periphery of an isolate was transferred to the center of each of 20 dishes. Radial growth from the center of the inoculum toward each well was measured weekly for 8 weeks. All cultures were incubated at $20^{\circ}\pm 2^{\circ}\text{C}$ under 3,400 lux of fluorescent light in growth chambers.

RESULTS

Proliferations of parenchymatous cells of the pine explants were visible to the unaided eye and easily seen through the polyethylene covers of the culture tubes or through the tube sides.

Citrus pectin caused the least impact on growth of the pine explants, with all explants showing proliferation in all treatments and only one explant exposed to 0.010 percent citrus pectin showing no growth (table 1). Late in the test period, 30 percent of the explants in the 0.1 percent treatment became brown, all of them in the 0.5 percent treatment became brown (table 2).

Table 1.--*Effects of pectic compounds on in vitro cell profusion of western white pine explants*

Treatment	Concentration (percent)						
	0.001	0.005	0.010	0.025	0.050	0.100	0.500
Citrus pectin	7/7*	7/7	6/7	7/7	7/7	7/7	7/7
Sodium polypectate	7/7	7/7	7/7	6/7	7/7	6/7	5/7
Pectic acid	7/7	7/7	6/7	7/7	5/7	1/7	0/7
HOH	1/7	0/7	0/7	0/7	0/7	0/7	0/7

*Number of explants showing increase in cell numbers/number of explants treated.

Table 2.--*Effects of pectic compounds on in vitro color of western white pine explants*

Treatment	Concentration (percent)						
	0.001	0.005	0.010	0.025	0.050	0.100	0.500
Citrus pectin	7/7*	7/7	6/7	7/7	7/7	3/7	0/7
Sodium polypectate	7/7	7/7	7/7	6/7	7/7	7/7	3/7
Pectic acid	6/7	7/7	5/7	7/7	5/7	0/7	0/7
HOH	1/7**	0/7	0/7	0/7	0/7	0/7	0/7

*Number of explants retaining a green color/number of explants treated.

**One callus of 49 survived, all others browned in 7-10 days.

Sodium polypectate inhibited cell proliferation in 14 percent of the explants at a concentration of 0.1 percent. Inhibition was evident in 29 percent of the 0.5 percent group. Color changes in this test group did not occur until the 0.5 percent level was reached and then 57 percent of the explants became brown.

Pectic acid had the most adverse effect on growth of cultured western white pine cells. Although no changes were noted in concentrations of 0.025 percent or less, 29 percent of the explants in the 0.05 percent treatment showed no proliferation and 40 percent of those showing cell multiplication had browned by the end of the test. Only 14 percent of the explants submerged under a 0.1 percent concentration of pectic acid had produced a visible increase in numbers of cells; however, at the end of the test all explants were brown. The 0.5 percent treatment, in addition to causing browning, inhibited growth of all explants.

Within the first to second week of exposure the blister rust fungus isolates began to respond to the pectic compounds. Both isolates exhibited suppressed but different growth rates, as shown in fig. 1. Sodium polypectate suppressed growth the least. Polygalacturonic acid, pectic acid, and citrus pectin were the most effective in retarding growth of both isolates with polygalacturonic acid being the most detrimental to the slow growing isolate.

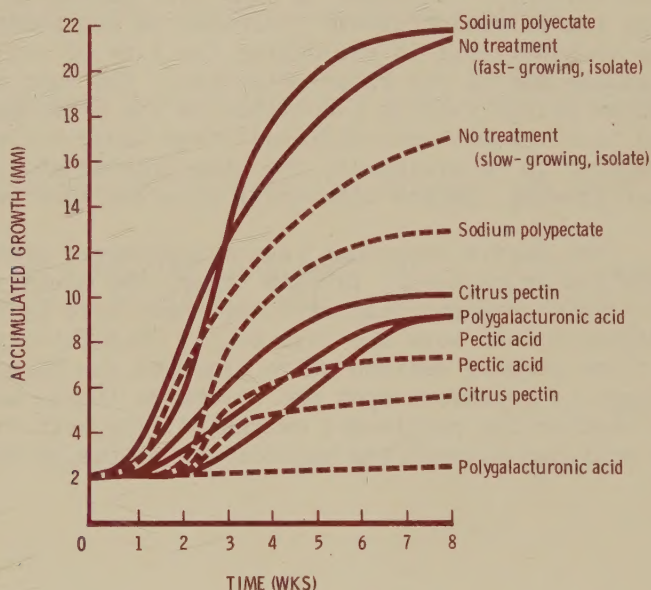


Figure 1.--Growth responses of two *Cronartium ribicola* isolates to pectin compounds.

DISCUSSION

The frequency of cell penetration by haustoria, wherever western white pine bark cells and the blister rust fungus make contact, implies that in a susceptible host-pathogen interaction products of enzymatic actions on native pectin are involved. In other studies, histochemical reactions and localized changes in electron density in the region of penetration suggest that carefully controlled enzymatic interactions between host and pathogen do occur. The extent of enzymatic alterations of cell wall pectins and other products generated by the invading pathogen are unknown.

The methyl esterases are perhaps the first to act upon pectin to expose host and pathogen to a low methoxy pectin compound similar to citrus pectin. Upon further activity, a compound void of methyl groups (represented by pectic acid) may be released. Both pine cells and rust mycelium responded negatively to such products. Pine parenchymatous cells began browning when exposed to a 0.1 percent concentration of citrus pectin, with all explants becoming brown at 0.5 percent. These cells were even more sensitive to pectic acid, becoming brown when exposed to 0.05 percent concentrations and showing complete color change and inhibition of proliferation with the 0.1 percent concentration. Apparently, the rust fungus can tolerate greater concentrations of these compounds than can its host because although growth of the rust was severely suppressed by the 1-percent concentrations, no symptoms of death, such as lysis or color change, were evident. The same responses were noted in the polygalacturonic acid treatment of the rust, i.e., growth was restrained and indicators of death were not evident.

The life of pine cells and fungus isolates was least affected by the highly water soluble sodium polypectate. The host cells showed a tendency toward color changes from green to brown only in the treatment with the highest concentration (0.5 percent). Increase in pine cell culture size was almost absent. This implies that the polygalacturonic chain may be detrimental to cell proliferation, but also may be beneficial in prolonging the life of cells, possibly as a pH buffer or as an exchange matrix for other molecules. The rust mycelium was the least affected by sodium polypectate in comparison to the other pectin compounds; however, growth rates and total growth were different from those achieved in control treatments. When compared to no treatment, the slow-growing isolate was slightly suppressed and the fast growing isolate apparently received some benefit.

The pectic compounds tested suppressed growth of both pine host cells and rust mycelium in culture. In this study, the hosts' cells succumbed at lower concentrations of pectic compounds and were more sensitive as the complexity of the pectic compounds decreased than were the rust cells. Deductively, then, in the host-parasite interactions between western white pine and the blister rust fungus, the survival of the fungus is dependent upon the tolerance limits of the host. It seems that, regardless of whether the pectinases originated from host or fungus, control of their activities is mediated through the physiologic status of the parasitized pine cells.

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Cronartium ribicola, the blister rust fungus of 5-needle pines establishes itself by adhering to the surfaces of host cells and will then penetrate the cell walls with haustoria. Success occurs when enzymes are produced that hydrolyze the cellulose, pectin, and protein constituents of the host cell and when the by-products of enzyme activity are not toxic to the host cell or fungus. Cultures of fungus and pine cells when challenged with pectin compounds responded through cell proliferation and death. Host's cells succumbed at lower concentrations of pectic compounds and were more sensitive as the complexity of the pectin compounds decreased than were the rust cells.

KEYWORDS: host-parasite interactions, blister rust, western white pine

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